Labeling of human mesenchymal stem cell: Comparison between paramagnetic and superparamagnetic agents

Chung-Yi Yang,[1,2] Ming-Fong Tai,[3] Shin-Tai Chen,[4] Yi-Ting Wang,[5] Ya-Fang Chen,[5] Jong-Kai Hsiao,[5] Jaw-Lin Wang,[2] and Hon-Man Liu[5,a)]

[1]*Department of Medical Imaging, National Taiwan University Hospital, Yun-Lin Branch, No. 579, Sec. 2, Yun-Lin Road, Douliou 640, Taiwan*

[2]*Institute of Biomedical Engineering, College of Medicine and Engineering, National Taiwan University, No. 1, Sec. 1, Jen-Ai Road, Taipei 100, Taiwan*

[3]*Department of Physics, National Tsing Hua University, No. 101 Sec. 2, Kuang Fu Road, Hsinchu 300, Taiwan*

[4]*Department of Biochemistry, Musculoskeletal Disease Center, J.L. Pettis VA Medical Center, Loma Linda University, Loma Linda, California 92357, USA*

[5]*Department of Medical Imaging, National Taiwan University Hospital and College of Medicine, 7 Chung-Shan South Road, Taipei 100, Taiwan*

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Paramagnetic and superparamagnetic substances are used to trace stem cell in living organisms under magnetic resonance imaging (MRI). We compared paramagnetic and superparamagnetic substance for their labeling efficiency by using clinically widely used gadolinium chelates and iron oxide nanoparticles. Without the aid of transfection agent, human mesenchymal stem cells were labeled with each agent separately in different concentration and the optimized concentration was determined by maintaining same cell viability as unlabeled cells. Iron oxide nanoparticle labeling has a detecting threshold of 12 500 cells *in vitro*, while gadolinium chelates labeling could be detected for at least 50 000 cells. In life animal study, we found there is an eightfold sensitivity in cells labeled with iron oxide superparamagnetic nanoparticles; however, the magnetic susceptibility artifact would obscure the detail of adjacent anatomical structures. We conclude that labeling stem cells with superparamagnetic substance is more efficacious. However, the cells labeled by superparamagnetic nanoparticles might interfere with the interpretation of anatomical structure. These findings would be beneficial to applications of magnetic substances toward stem cell biology and tissue engineering. © *2009 American Institute of Physics*. [DOI: 10.1063/1.3072821]

I. INTRODUCTION

Mesenchymal stem cell (MSC) is a specific type of multipotent stem cell that is capable to differentiate into bone, muscle, cartilage, fat, and other tissues. The current therapeutic applications of MSC include gene therapy and tissue engineering through local delivery or systemic infusion transplantation.[1,2] Once stem cells are delivered into the human body, it is very important to follow the existence, the growth or proliferation of the cells, and to trace its migration between different organs.[3] With the current methodologies, it is difficult to study these parameters *in vivo*.

Magnetic resonance imaging (MRI) has much more flexible intrinsic contrast than any other clinical imaging modality. The administration of magnetic contrast agents can modify intrinsic magnetic parameters and provides greater contrast between normal and diseased tissue.[4] In molecular and cellular imaging, paramagnetic and superparamagnetic substances are used as contrast agents for labeling stem cells so that the cells could be visualized on MRI.[5] However, the efficacy, sensitivity, and feasibility between paramagnetic and superparamagnetic substances labeling were not fully investigated. Moreover, most of the labeling required transfection agent that might influence the cell behavior. We previously developed cell labeling without transfection agent and in this study, human MSCs (hMSCs) labeled with paramagnetic and superparamagnetic materials were tested to evaluate the influence on the cells and the MRI characters of these substances.

II. EXPERIMENT

In order to check the magnetic properties of gadodiamide, magnetic hysteresis measurements at room temperature were carried out by using a Quantum Design MPMS SQUID magnetometer with the applied magnetic field up to 3 T. The magnetic properties of ferucarbotran were measured previously.[6]

hMSCs immortalized by gene transfer of combination of human telomerase reverse transcriptase with human papilloma virus E6 and E7 were cultured as described.[7] hMSCs were incubated with either superparamagnetic substance, ferucarbotran (Resovist, Schering AG, Berlin, Germany; or paramagnetic substance, gadodiamide (Omniscan, GE Healthcare, Waukesha, Wisconsin, U.S.; $R1/R2 = 3.9/4.3\ s^{-1}\ mM^{-1}$) nanoparticles of different concentration for the following examination.

For the labeling of hMSCs, the cells were seeded with 1×10^5 cells per well in six-well plates and were allowed to

a)Author to whom correspondence should be addressed. Electronic mail: hmliu@ntu.edu.tw.

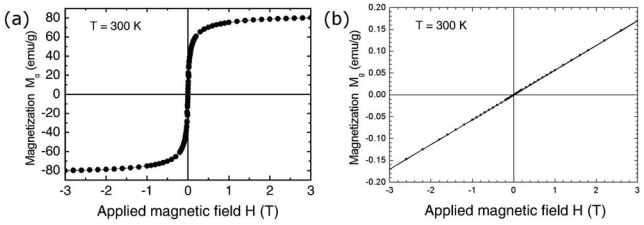

FIG. 1. Magnetization curves of ferucarbotran (a) and gadodiamide (b).

attach for 24 h. After 24 h of incubation of ferucarbotran or gadodiamide in different concentrations without any transfection agent, flow cytometry was used to determine the cell size and granularity change.[7] Reactive oxygen species (ROS) production of hMSCs after particles administration was measured with flow cytometry using a dichlorofluorescein diacetate fluorescent probe.[8] Mitochondria membrane potential (MMP) in hMSCs was measured with fluorescent intensities analyzed by flow cytometry.[9] MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used with a microplate reader to evaluate cell viability and proliferation.[7]

The enhancement of untreated and a series of diluted labeled 1000 to 2×10^5 hMSCs were evaluated using two-dimensional T2-weighted gradient echo pulse sequence with a clinical 1.5 T MR System (Signa Excite, GE Healthcare, USA) and an eight-channel head coil.[10] Male BALB/c mice (8 weeks of age) were obtained from National Laboratory Animal Center, Taiwan. The labeled cells (1×10^5 cells/100ul) were injected subcutaneously into the dorsal thighs of the mice. Five hours later, animal MRI studies were performed under intraperitoneal anesthesia. The mice were then scanned with a custom-made radiofrequency coil in the same clinical 1.5 T MR system as *in vitro* study.[10]

III. RESULTS AND DISCUSSION

A. Cell culture and flow cytometry detection

Gadodiamide revealed linear increase in magnetism that comply with paramagnetic substance. Under 3 T magnetic field, we measured 0.17 emg/g. The ferucarbotran revealed superparamagnetism with satuation magnetization of 80 emu/g and the Hc is zero (Fig. 1). We first tested the toxicity of Gadodiamide with 0.95, 2.87, 9.53, or 28.7 mg/ml in hMSCs. We found mass cells death with 28.7 mg/ml of gadodiamide (data not shown). Further analysis will be required to elucidate the toxicity of gadodiamide at this concentration. Apoptosis has been observed in cells incubated with high iron concentrations 500 μg Fe/ml.[11] We did not observe any cells death or morphological change in hMSCs with 9.53 mg/ml of gadodiamide, nor for hMSCs with 10 μg/ml of ferucarbotran under light microscopy [Figs. 2(a)–2(c)]. Prussian blue staining of ferucarbotran-labeled hMSCs also confirmed the uptake of iron content [Fig. 2(c)].

Flow cytometric measurement confirmed that the labeling of ferucarbotran or gadodiamide did not influence the forward and side light scattering (FSC and SSC, respectively) of the stem cells [Fig. 2(d)]. The result suggests that the concentration we used for labeling does not significantly influence the cell size and granularity. The production of

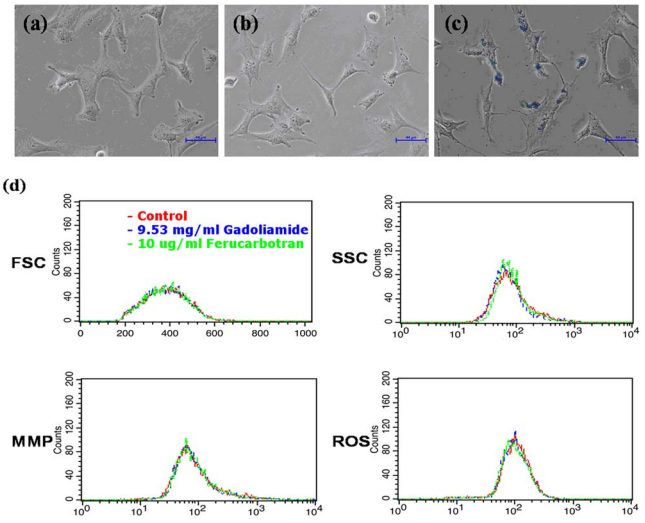

FIG. 2. (Color online) Light microscopic appearance of hMSCs [(a)–(c)] and results of flow cytometry. There is no significant alteration of cell size and morphology after labeling of gadodiamide (b) or ferucarbotran (c) compared with unlabeled hMSCs (a). (d) Flow cytometric measurement of cells treated with different agents. The measurement of FSC and SSC was done 3 days after incubation with specific agents. The measurement of ROS and MMP was done after 24 h of incubation with each agent.

ROS can result in lipid peroxidation and DNA damage and ultimately causes toxic effect on cells. The ROS production were not significantly altered in both paramagnetic and superparamagnetic substance-labeled hMSCs when compared with the unlabeled cells [Fig. 2(d)]. Mitochondrial permeability transition is a central coordinating event of apoptosis, and a number of apoptotic phenomena are triggered after that.[9] In this study [Fig. 2(d)], there was no evidence of compromised hMSCs viability when ferucarbotran at the concentration of 10 μg/ml or gadodiamide at the concentration of 9.53 mg/ml was used.

B. Cell viability and proliferation

As shown in Fig. 3, 4-hr or overnight treatment did not result in significant difference in cell viability and it is less than 10% difference as compared with the unlabeled cells.

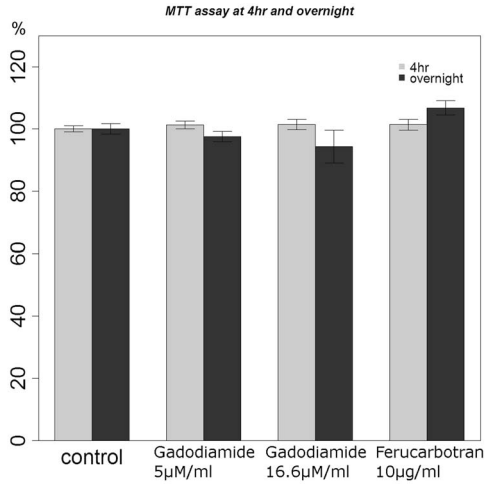

FIG. 3. (Color online) Result of MTT assay after 4 h or overnight of magnetic particles labeling of hMSCs.

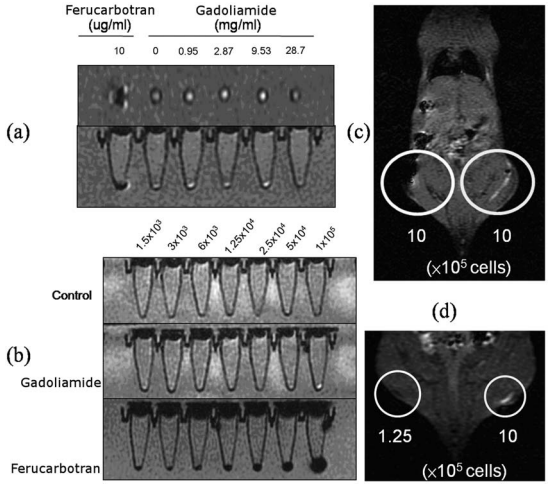

FIG. 4. *in vitro* and *in vivo* MR imaging of hMSCs. Part (a) shows two different sections of the MR imaging of 2×10^5 labeled cells that have incubated with different concentration of magnetic particles. Gradual increase in intensity is noted with the increase of the gadodiamide concentration from (0 to 9.53 mg/ml). The signal intensity of 28.7 mg/ml gadodiamide labeling decreased as compared with that of 9.53 mg/ml gadodiamide labeling (a). Part (b) shows the MR imaging of different cell number (from 1500 to 1×10^5) incubated with either 9.53 mg/ml gadodiamide or 10 μg/ml ferucarbotran. Part (c) is MR imaging of labeled hMSCs in a BALB/c mouse. (d) The minimal number of labeled hMSCs that could be detected in life animals by clinical MRI. There is an eightfold sensitivity in cells labeled with iron oxide superparamagnetic nanoparticles.

The results suggest no significant alteration in MTT after labeling of each magnetic particle in these concentrations.

C. *In vitro* MR imaging of labeled cell

First, we compared the labeling of 2×10^5 cells of hMSCs with different amount of gadodiamide and 10 μg/ml of ferucarbotran. As shown in Fig. 4(a), we found that the labeled cells can be detected even at the lowest concentration of gadodiamide. Interestingly, the signal of hMSCs with the highest concentration of gadodiamide is very low and this may due to the mass cells' death labeling. Second, we used different numbers of hMSCs labeled with gadodiamide and ferucarbotran under the optimal condition. [Fig. 4(b)]. We found that gadodiamide-labeled hMSCs only can be detected by MRI when cell number is greater than 50 000, conversely, ferucarbotran-labeled hMSCs can be detected by MRI with only 12 500 cells in the tube.

As expected, we could find that ferucarbotran (as a negative contrast agent) caused signal intensity drop not only at the cells themselves but also the surrounding areas on the MRI. The profound dark signal produced by the superparamagnetic material on MRI obscured the contour of the test tube. On the contrary, cells labeled with the gadodiamide, a positive contrast agent in this case, caused increased signal intensity but only within the cells on the MRI [Figs. 4(a) and 4(b)], which provides good contrast between hMSCs and their surrounding environment.

D. MR imaging of labeled hMSCs in BALB/c mice

In life animal study, we first injected equal numbers of different labeled cells to each thigh of mice. The side injected with iron oxide superparamagnetic nanoparticle labeled cells appears as profound dark signal at the left thigh [right circle in Fig. 4(c)] while the paramagnetic gadolinium complex caused increased signal in intermuscular spaces, as shown in Fig. 4(c) (left circle). The affected area of MRI is larger in ferucarbotran-labeled cells than in gadodiamide-labeled cells, however, the magnetic susceptibility artifact caused by paramagnetic nanoparticles interrupted the visualization of adjacent structures. We also determined the minimal number of labeled hMSCs that could be detected in life animals by clinical MRI. As shown in Fig. 4(d), we can use 1.25×10^5 of ferucarbotran labeled cells to achieve similar signal with 1×10^6 of gadodiamide labeled cells. The higher MRI sensitivity of superparamagnetic labeling is especially useful when the injected stem cells are small in number. On the other hand, to delineate the growth of the injected stem cells, we could label the cells with paramagnetic particles. The property of positive contrast agent makes it suitable for labeling stem cells implanted into organs that appear dark on MRI (e.g., lungs). On the other hand, labeling with the negative contrast agent can be used in organs that appear as high signal intensity, for example, kidney or lymphoid tissues. The understanding of signal alteration by paramagnetic and superparamagnetic substances helps physicians to label the stem cells properly on target organ to be salvaged.

IV. CONCLUSION

We conclude that the labeling with specific dose of paramagnetic and superparamagnetic substances does not alter the morphology, cell size, granularity, MMP, MTT, or ROS production of the hMSCs. MRI detection of stem cells labeled with superparamagnetic substance is more efficacious and more sensitive, but in case the evaluation of organ structure is critical, paramagnetic labeling is more favored. These findings would be beneficial for development of stem cell biology and tissue engineering.

ACKNOWLEDGMENTS

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